# Host-specific differences in the contribution of an extended spectrum β-lactamase (ESBL) IncI1 plasmid to intestinal colonisation by Escherichia coli O104:H4

Giles, Michaela; Cawthraw, Shaun; AbuOun, Manal; Thomas, Christopher; Munera, Diana; Waldor, Matthew; La Ragione, Roberto; Ritchie, Jennifer

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**Table S1. Bacterial strains and primers used in this study.**

| Strain/ plasmid | Genotype/phenotype | Reference |
|---|---|---|
| BL211 | *E. coli* O104:H4 Δ*stx2AB::*Gent$^{R}$; Tet$^{R}$; Ctx$^{R}$ | [5] |
| BL320 | BL211 derivative lacking pESBL | This study |
| pIMF27 | Inc1 curing vector, Kan$^{R}$ | [13] |

Primers

| Gene | Sequence | Reference |
|---|---|---|
| *Chromosomal markers* | | |
| *terD* (434 bp) | 5'-AGTAAAGCAGCTCCGTCAAT-3'<br>5'-CCGAACAGCATGGCAGTCT-3' | [3] |
| *rfb*$_{O104}$ (351 bp) | 5'-TGAACTGATTTTTAGGATGG-3'<br>5'-AGAACCTCACTCAAATTATG-3' | [3] |
| *fliC*$_{H4}$ (201 bp) | 5'-GGCGAAACTGACGGCTGCTG-3'<br>5'-GCACCAACAGTTACCGCCGC-3' | [3] |
| *Plasmid-specific markers* | | |
| *aggR* (254 bp) for pAA | 5'-GTATACACAAAAGAAGGAAGC-3'<br>5'-ACAGAATCGTCAGCATCAGC-3' | [37] |
| *bla*$_{CTX-M}$ (593 bp) for pESBL | 5'-ATGTGCAGYACCAGTAARGTKATGGC-3'<br>5'-TGGGTRAARTARGTSACCAGAAYAAGCGG-3' | [38] |
| *alternative bla*$_{CTX-M}$ (260 bp) | 5'-GCGTGATACCACTTCACCTC-3'<br>5'-TGAAGTAAGTGACCAGAATC-3' | [39] |

PCR reactions were performed using the QIAGEN Multiplex PCR reagents and the following conditions: 95˚C for 15 min, followed by 25 cycles consisting of 95 ˚C for 30s, 54 ˚C for 30s and 72 ˚C for 1 min, with a final extension step of 72 ˚C for 5 min. PCR products were analysed by electrophoresis on 2% agarose gels and visualised using ethidium bromide (5 µg/mL) or Redsafe$^{TM}$.

**Table S2. Diarrhoeal status of infant rabbits infected with the different strains of bacteria.**

| Strain | BL211 | BL320 |
|---|---|---|
| Diarrhoea (%) | 18 | 11 |
| Disease category | | |
| Diarrhoea | 0 | 0 |
| Intestinal disease | 3 | 2 |
| None | 14 | 17 |
| Total number of animals | 17 | 19 |
| P-value* versus BL211 | - | NS |

*Fisher's exact test

**Table S3. Recovery of *E. coli* O104 strains in weaned sheep at 4 days post infection.**

| Strain | Animal No. | cfu/g tissue sample [$log_{10}$] | | | | |
|---|---|---|---|---|---|---|
| | | ileum | caecum | colon | rectum | RAJ[a] |
| BL211 | 1 | 0[b] | 2.5 | 2.8 | 0 | 0 |
| | 2 | 0 | 1[c] | 0 | 1 | 1 |
| | 3 | 5.0 | 4.2 | 3.9 | 1 | 1 |
| BL320 | 1 | 0 | 2.3 | 2.3 | 2.8 | 1 |
| | 2 | 2.3 | 1 | 1 | 1 | 2.0 |
| | 3 | 0 | 1 | 1 | 1 | 2.3 |

RAJ[a] = recto-anal junction

0[b] = No colonies were recovered even after enrichment

1[c] = Colonies were recovered after enrichment

**Figure S1**

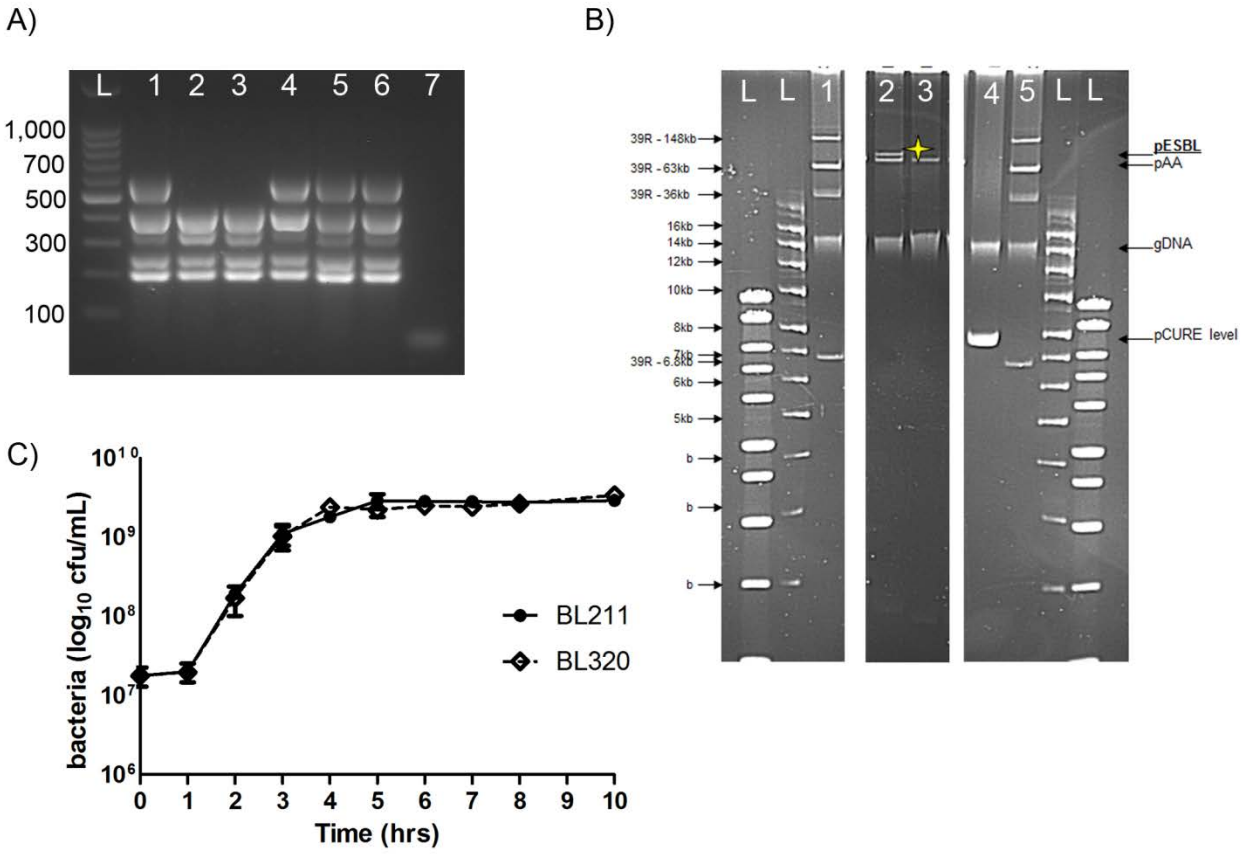


**Figure S1. Confirmation of pESBL loss from *E. coli* O104:H4.** Multiplex PCR was used to amplify chromosomal (*terD* (434bp), $rfb_{O104}$ (351 bp), *fliC* (201 bp) and plasmid-borne ($bla_{CTX\text{-}M}$ (593 bp), *aggR* (254 bp)) genes (A). Products were visualised on a 2% agarose gel. Lanes: L - 100 bp DNA ladder, 1- parent strain BL211; 2 - pESBL-cured strain BL320; 3 – cefotaxime-sudceptible output colony; 4 to 6 – representative cefotaxime-resistant colonies and 7 – negative control ($ddH_2O$). Plasmid profile of strains (B). Plasmid DNA and visualised on 0.8% agarose gel. Lanes: L-DNA ladders; 1 – *E. coli* 39R reference strain; 2 – wild type strain BL211; 3 – pESBL-cured strain BL320; 4 – pIFM27 plasmid; 5 – *E. coli* 39R reference strain. All lanes are from the same gel but extra lanes have been removed. Yellow star indicates missing band corresponding to pESBL plasmid. Growth of wild-type and pESBL-cured strain in LB media over time (C). Bacterial numbers were determined by serial dilution and plating

on LB agar supplemented with antibiotics. Data represent means +/- standard deviation of 3 biological replicates.